Journal of Agricultural Technology 2011 Vol. 7(2): 431-439
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Characterization of *Phytophthora infestans* population in potato crops from Chiang mai and Tak provinces

Jaimasit, P. and Prakob, W.*

Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand 50200.

Jaimasit, P. and Prakob, W. (2011). Characterization of *Phytophthora infestans* population in potato crops from Chiang mai and Tak provinces. Journal of Agricultural Technology 7(2): 431-439.

A total of 117 isolates of *P. infestans* were isolated from blighted potato foliage. The isolates were obtained from major potato-growing areas in Chiang Mai and Tak provinces between 2006 and 2009. These isolates were analyzed for their mating type, resistance to metalaxyl and mtDNA haplotypes. The results showed that all of these isolates are mating type A1 and most are susceptible to metalaxyl, with 27 isolates being metalaxyl intermediate and 11 isolates are resistant to metalaxyl. In addition, one of four mtDNA haplotypes, IIa, dominated the population. This finding suggests limited diversity within the current studied field population of *P. infestans* in Chiang Mai and Tak provinces. However, to gain a better understanding of structure and biodiversity among *P .infestans* populations in Thailand, these isolates as well as isolates acquired from other areas should be further genotypically characterized by using additional molecular techniques.

Key words: Late blight, *Phytophthora infestans*, metalaxyl resistance, mating type and population genetics.

Introduction

Phytophthora infestans is an oomycete responsible for the late blight diseases found in potatoes and tomatoes. First appearing in the 1840s as the cause of the Irish potato famine, late blight has become a particularly devastating disease worldwide during the past few decades (Goodwin *et al*., 1994a; Goodwin *et al*., 1994b). The pathogen is heterothallic and forms oospores between A1 and A2 mating types (Galindo and Gallegly, 1960; Judelson, 1997). Until the 1980s, the global population of *P. infestans* outside of central Mexico was thought to be derived from a single A1-mating-type

*Corresponding author: Waraporn Prakob; e-mail: awarapornp@hotmail.com

clonal lineage, the 'old' population known as the US-1 lineage (Goodwin *et al*., 1994b). However, since then, isolates of 'new' populations of both A1 and A2 mating types have been found in other areas around the world (Hohl and Iselin, 1984; Deahl *et al*., 1991). Some of these isolates were shown to have metalaxyl resistance and a broader range of virulence factors (Dowley and Sullivan, 1981). The genetic and phenotypic diversity of *P. infestans* populations has subsequently been investigated in many countries (Goodwin *et al*., 1994a; Goodwin *et al*., 1995c; Koh *et al*., 1994; Day *et al*., 2004). These studies suggested that new populations of *P. infestans* appear as a result of migration into regions and / or sexual recombination within them. A global marker database for *P. infestans*, containing information on RFLPs obtained using the RG57 probe, mitochondrial DNA (mtDNA), haplotypes (Carter *et al*., 1990; Goodwin, 1991) allozyme genotypes, sensitivity to metalaxyl, mating types and other factors, was compiled (Forbes *et al*., 1998) Following the construction of the database, some reports of Asian *P. infestans* populations were published. Isolates found in Korea, India, Taiwan, Indonesia, Thailand, Nepal and China during the period from 1992–1997 were also investigated and the results showed A2-mating-type isolates and Asian-specific allozyme genotypes. In Thailand, potato is usually grown in a single winter crop grown primarily from October to February in Northern and North-eastern part of the country. Growing areas of potato tend to increase every year to provide potato to food industry. Even though, the late blight epidemics have not been well recorded in Thailand, occurrence of the disease is found every year, often causing heavy economic losses. In addition to losses suffered due to reduced yields, growers have incurred the further expense of the many fungicide applications required to manage the disease. Phenylamide fungicides, especially metalaxyl, were the most effective and most commonly used fungicides against late blight. There is evidence which shows that isolates of 'new' populations of both A1 and A2 mating types, some of which are metalaxyl resistant (Dowley and Sullivan, 1981) and have a broader range of virulence factors (Smoot *et al*., 1958) have been found in many parts of the world (Hohl and Iselin, 1984; Deahl *et al*., 1991). To establish the most effective control strategies for late blight, it is neccessary to have up-to-date information on dispersal and variation within local *P. infestans* populations. Therefore, the appearance and rapid spread of metalaxyl-resistant strains of *P infestans* isolates found in Thailand should be thoroughly investigated. Moreover, studying the genetic and phenotypic structure of the *P. infestans* population in Thailand is required to provide an accurate understanding of the population structure and gene flow between *P. infestans* populations. This study aims to characterize the population of *P. infestans* isolates, collected from various potato-cultivating areas in Chiang Mai

and Tak provinces, by determining their mating type, mitochondrial DNA (mtDNA) haplotypes and resistance to the chemical Metalaxyl.

Materials and methods

Sampling of P. infestans isolates

Isolates of *P. infestans* with natural late blight infections were obtained from six major potato-growing areas of Chiang Mai and Tak provinces, including Praow, Sansai and Pobpra districts. The isolates were collected during potato growing seasons between 2006 and 2009 and the blighted potato leaves, with freshly sporulating lesions of *P. infestans*, were collected from fields which were separated by at least several kilometers. The samples were then transported in plastic bags to the laboratory.

Isolation of P. infestans

To promote sporulation, the infected potato leaflets were placed in a plastic box containing moist filter paper and incubated in darkness at 18°C for 24 hours. The leaflets, with freshly formed sporangia were then pressed briefly against selective media amended with antibiotics (ampicillin 50 µg/ml, nystatin 100 µg/ml, rifampicin 50 µg/ml and benomyl 10 µg/ml). The plates were then incubated at 18^{o}C for 5-7 days to allow mycelia to grow into the medium. Small agar blocks containing hyphal tips were then cut from the colony margins and transferred to new amended rye A agar three more times before being maintained on unamended rye A agar.

Mating type differentiation

Mating type was determined by placing an unknown isolate 2.5 cm between two known A2 tester isolates of *P. infestans* (E13 and 618, which had been isolated from Egypt and Mexico, respectively) on Rye A agar media. Hyphal interaction zones were observed microscopically after 7 days of incubation at 18°C in darkness. Oospores were produced in the margins of opposite mating types. Isolates that produced oospores with the known A2 tester isolates were designated as the A1 mating type and isolates that did not produce oospores with the known A2 tester isolates were designated as the A2 mating type. Self-fertile isolates were also examined by examining 10 blocks of Rye A agar on which each isolate was grown for oospore production. Since there is no standard A1 used in this study, the isolates designated as mating A2 (as no oospore formation was observed when mated with standard mating A2)

were mated again with the mating type A1 isolates which are identified in this study. Duplicate mating type tests were performed.

Metalaxyl resistance

All isolates of *P. infestans* were tested for phenylamide fungicide metalaxyl resistance *in vitro*. The EC50 values were calculated on the basis of growth inhibition on rye. A agar amended with four concentrations (0, 5 and 100 mg/l) of metalaxyl. The isolates were assigned to one of three groups: sensitive (EC50<5 mg/L), intermediate (5 mg/L<EC50< 100 mg/l) or resistant (EC50> 100 mg/l). Three replicates were used for each isolate.

MtDNA haplotypes

Mitochondrial DNA (mtDNA) haplotypes of 117 isolates were identified by using the method previously described by Griffith and Shaw (1998). DNA extraction was made by Nucleospin kit (Macherey-Nagel Inc. PA, USA). The primer pairs used to amplify region P2 and P4 were F2+R2 and F4+R4, respectively.

F2 (5'- TCCCTTTGTCCTCTACCGAT -3')
R2 (5' -TTACGGCGGTTTAGCACATACA -3')
F4 (5'-TGGTCATCCAGAGGTTTATGTT -3')
R4 (5'- CCGATACCGATACCAGCACCAA -3')

The polymerase chain reaction (PCR) products of P2 and P4 were digested with *Hpa*II instead of *Msp*I (Griffith and Shaw, 1998) which digests the same site, and *Eco*RI, respectively, and analysed by 2% agarose gel electrophoresis.

Results

Isolation of P. infestans

Due to unfavorable climate conditions during the winter season (Dec. 2006-Feb. 2009), occurrences of the late blight epidemic were extremely limited. A total of 117 isolates of *P. infestans* were isolated from infected potato leaves collected from two major potato-cultivating areas in Chiang Mai and Tak provinces. Pure cultures of all isolates were obtained by culturing sporangia on a selective medium amended with antibiotics as described previously in the materials and methods portion of this document. The colony morphology (white and fluffy) is similar in all *P. infestans* isolates. The morphology of sporangia and mycelia was also observed under a compound microscope and the results showed nonseptate sporangia on obvious sporangiophore structures whose morphology matched that

of *P. infestans*. Sporangia were an average of 45 µm in length and 27 µm in width with a length/breadth ratio of 1.66. Sporangia were caducous and limoniform to ovoid in shape.

Mating type and sensitivity to metalaxyl

All isolates were proved to be A1 mating type as thick-walled sexual spores called oospores were produced during mating between these isolates with both standard A2 isolates, E13 and 618. The produced thick-walled oospores have antheridia attached around the oogonial stalk (amphigynous). No self-fertile isolates were found in this study. In *vitro* assays for metalaxyl sensitivity indicated that most isolates collected between December 2006 and February 2009 were metalaxyl sensitive with 27 and 11 isolates which are metalaxyl intermediate and resistant, respectively (Table 1).

Mitochondrial DNA haplotype

The amplification product sizes obtained from both regions (P2 and P4) of mitochondrial DNA were similarly with those obtained by Griffith and Shaw (1998). After digestion of PCR products with restriction enzymes, the size of restriction fragments are similar to those obtained by Griffith and Shaw (1998). There are three restriction fragments (720, 203 and 147 bp) were obtained after cutting P2 amplified product with *HpaII* (Fig.1) whereas two restriction fragments (603 and 361 bp) were obtained after P4 amplified product was cut with *EcoRI* (Fig.2). This finding indicated that all the 117 isolates are IIa haplotype.

Table 1. Characteristics of *Phytophthora infestans* isolates collected from potato crops in Chiang Mai and Tak provinces between 2006 and 2009.

Region	Sampling year	No. of fields	No. of isolates	Mating type		Metalaxyl[b] resistance			mtDNA haplotype			
				A1	A2	S	I	R	Ia	Ib	IIa	IIb
Sunsai, CM[a].	2006	5	61	61	0	58	3	0	0	0	61	0
Praow, CM.	2007	1	20	20	0	19	1	0	0	0	20	0
Sunsai, CM.	2009	1	15	15	0	2	11	2	0	0	15	0
Pobpra, Tak	2009	1	21	21	0	0	12	9	0	0	21	0

[a]Chiang Mai., [b]Tak, [c]S, I, and R denote sensitive, intermediate and resistant to metalaxyl, respectively.

Discussion

The presence of two mating types of heterothallic isolates of *Phytophthora infestans* is a prerequisite for their sexual reproduction and their mating type has been used as an indication of the origin of the (Galindo and Gallegly, 1960; Koh *et al*., 1994). Before 1984, the A2 mating type had only been found in Mexico, which was widely accepted as the possible origin of *P. infestans*. The A1 mating type prevailed throughout the rest of the world, including the United States, Canada, Western Europe, South Africa, and West India (Smoot *et al*., 1958). In recent years, the A2 mating type has been detected in many parts of the world. In 1984, it was first reported in Switzerland and subsequently discovered throughout Europe, North America and Asia. This suggests that migration was the cause of the new occurrences of the A2 mating type. Long-distance migration of *P. infestans* frequently appears to have resulted from the inadvertent movement of infected plant material (potato tubers, tomatoes), an unintended result of international trade. The appearance of new populations of *P. infestans* has often been accompanied by devastating results: loss of resistant varieties of hosts, the appearance of fungicide-resistant strains (Semal, 1995; Shaw, 1987; Sujkowski *et al*., 1994) and a broader range of virulence factors (Drenth *et al*., 1994). Tomatoes and potatoes are significant vegetable crops in Thailand, both of which are vulnerable to late blight disease. In the current study, a total of 117 pure isolates of *P. infestans* were attained from infected potato leaves. The isolates of *P. infestans* tested in this study all proved to be of the A1 mating type. These results are similar to the study by Nishimura *et al* (1999). Pechaboon (2003)) in that A1 mating type isolates were found in the districts of Mae-rim, Mae-Tang, Sunsai and Praow, which are located in Chiang Mai. However, the A2 mating isolates were only found in Chaiprakran and Fang districts which are also located in Chiang Mai (Nishimura *et al*., 1999). This finding indicated there is no change in the mating types of the *P. infestans* populations in Sunsai and Praow districts. The isolates studied by Gotoh *et al* (2005), in which from a total of 44 isolates collected in Chiang Mai in 1994, 22 mating type A2 isolates were found, however the specific origin of these isolates was not given (Gotoh *et al*., 2005). Most isolates in this finding were metalaxyl sensitive, with the exception of 27 isolates which were metalaxyl intermediate, with 11 isolates being resistant. In contrast, all the isolates reported by Nishimura *et al*., 1999 and Gotoh *et al* (2005) were sensitive to metalaxyl. Pechaboon (2003) also reported that most of isolates collected in Sunsai and Praow districts were resistant to metalaxyl. That is different from our result in that most of the isolates incollected from the Sunsai and Praow districts are susceptible to metalaxyl. This might be because of the difference in the fields of areas in

which the isolates were collected. Also the application of the chemical metalaxyl in the areas where the isolates were collected in this study is less or these areas might have just been developed to cultivate potato crop. Our result is also similar to the findings by Petchaboon (2003) in that most of the Thai *P. infestans* that are resistant to metalaxyl in this study were collected from potato fields located in the Pobpra district, where aggressive use of chemicals is prevalent. It is possible that resistance to the systemic fungicide metalaxyl was a result of long term and sequentially increased concentrated use of metalaxyl (Dowley and Sullivan, 1981).). It was also found in this study that almost all 117 isolates have mtDNA haplotype IIa. Griffith and Shaw (1998) addressed that haplotype Ia was most often associated with A1, and haplotype IIa was most often associated with A2 mating type, however Ia haplotype was not found in this study. Combining all of the results suggests that there is low diversity among *P. infestans* populations. However, this current study only provides preliminary data of the *P. infestans* population in Thailand. Further work is needed to establish a complete structure of the entire population as well as gene flow between *P. infestans* populations. Moreover, to determine whether the Thai *P. infestans* population belongs to the old or new type and if there are any Thai and other Asian specific genotypes or recombinant genotypes among the populations, it will be necessary to characterize isolates used in this study and other isolates collected from potato and / or tomato fields from other areas. By using additional markers such as RFLP profiles, obtained by using the RG57 probe, mitochondrial DNA (mtDNA) haplotypes, allozyme genotypes and other factors, will be compiled.

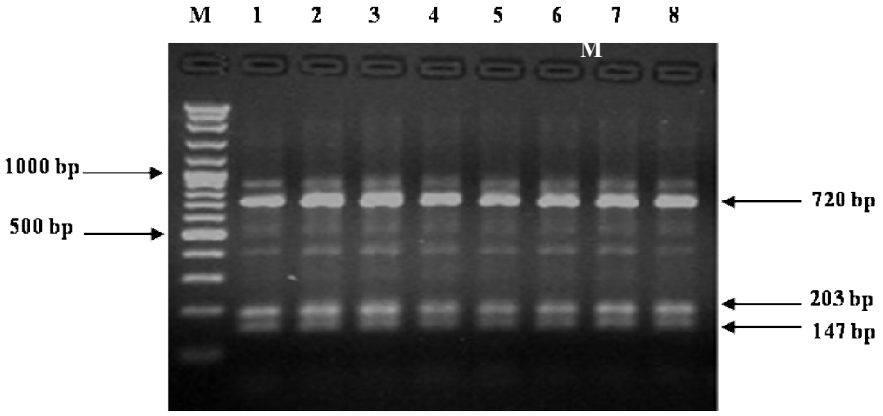

Fig. 1. Restriction enzyme digestions of PCR products amplified from *P. infestans*' mtDNA with primer pair F2-R2 (cut with *Hpa*II). Amplifications were conducted with DNA from a representative isolate of each field. Lane marked M contains 100-bp ladder (Vivantis Ltd.).

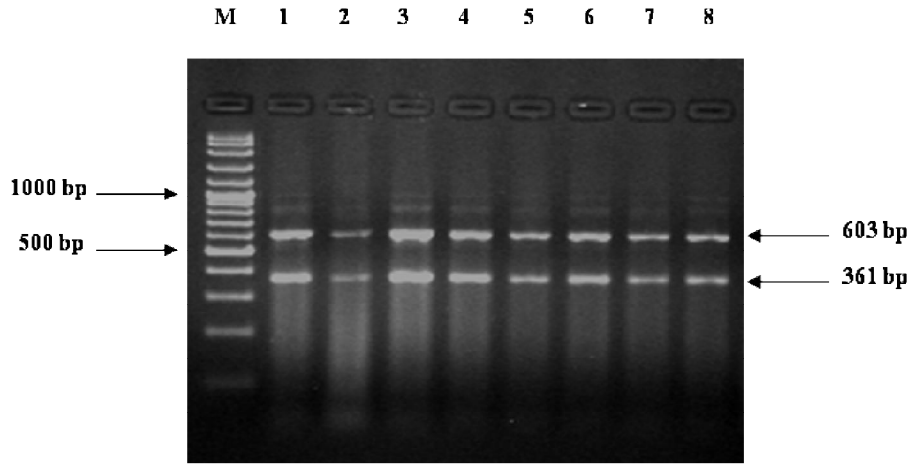

Fig. 2. Restriction enzyme digestions of PCR products amplified from *P. infestans*' mtDNA with primer pair F4-R4 (cut with *Eco* RI). Amplifications were conducted with DNA from a representative isolate of each field. Lane marked M contains 100-bp ladder (Vivantis Ltd.).

Acknowledgements

This work has been financially supported by Chiang Mai University (awarded in 2009 for young investigators) and also the Graduate School, Chiang Mai University. We are grateful to Prof. Howard S Judelson, Dept. of Plant Pathology, University of California, Riverside, for providing helpful advice and the strains of A2 mating type. We also thank the Central lab, Faculty of Agriculture, CMU for providing the lab facility. We would also like to thank Greg T. Sholly for editing the manuscript.

References

Carter, D.A., S.A. Archer, K.W. Buck, D.S. Shaw and R.C. Shattock. (1990). Restriction fragment length polymorphisms of mitochondrial DNA of *Phytophthora infestans*. Mycological Research 94: 1123–1128.

Day, J.P., R.A.M. Wattier, D.S. Shaw and R.C. Shattock. (2004). Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britiain, 1995–1998. Plant Pathology 53: 303–315.

Deahl, K.L., R.W. Goth, R.Young, S.L. Sinden and M.E. Gallegly. (1991). Occurrence of the A2 mating type of *Phytophthora infestnas* in potato fields in the United States and Canada. American Potato Journal 68: 717–726.

Dowley, L.J. and E. O'Sullivan. (1981). Metalaxyl-resistant strains of *Phytophthorainfestans* (Mont.) de Bary in Ireland. PotatoResearch 24: 417–421.

Drenth, A., I.C.Q. Tas and F. Govers. (1994). DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. European Journal of Plant Pathology 100: 97–107.

Forbes, G.A., S.B. Goodwin, A. Drenth, P. Oyarzun, M.E. Ordonez and W.E. Fry. (1998). A global marker database for *Phytophthora infestans*. Plant Disease 82 : 811–818.

Galindo, J.A. and M.E. Gallegly. (1960). The nature of sexuality in *Phytophthora infestans*. Phytopathology 50: 123–128.

Griffth, G.W. and D.S, Shaw. (1998). Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected after PCR amplication of DNA from pure cultures or from host lesions. Applied and Environmental Microbiology 64: 4007-4014.

Gotoh, K., S. Akino, M. Kato, N. Kondo and S. Naito. (2005) *Characterization of some Asian isolates of* Phytophthora infestans. Plant Pathology 54: 733–739.

Goodwin, S.B., B.A. Cohen, K.L. Deahl and W.E. Fry. (1994a). Migration from northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. Phytopathology 84: 553–558.

Goodwin, S.B., B.A. Cohen and W.E. Fry. (1994b). Panglobal distribution of a singleclonal lineage of the Irish potato famine fungus. Proceedings of the National Academy of Sciences,USA 91: 11591–11595.

Goodwin, S.B., L.S. Sujkowski and W.E. Fry. (1995c). Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. Phytopathology 85: 669–676.

Goodwin, S.B. (1991). DNA polymorphisms in *Phytophthora infestans*: the Cornell experience. In: Lucas JA, Shattock RC, Shaw DS, Cooke LR, eds. Phytophthora. Cambridge, UK: Cambridge University Press: 256–271.

Hohl, H.R., K. Iselin. (1984). Strains of *Phytophthora infestans* from Switzerland with A2 mating type behavior. Transactions of the British Mycological Society 83 : 529–530.

Judelson, H.S. (1997). The genetics and biology of *Phytophthora infestans*: modern approaches to a historical challenge. Fungal Genetics and Biology 22 : 65–76.

Petchaboon, K. (2003). Characterization of Population Structure of *Phytophthora infestans* from Tomato and Potato in Tak and Chiang Mai Provinces. Master Thesis, Department of Plant Pathology, Faculty of Agriculture, Kasedsart University, Thailand. 107 pages.

Koh, Y.J., S.B. Goodwin, A.T. Dyer, B.A. Cohen, A. Ogoshi, N. Sato and W.E. Fry. (1994). Migrations and displacements of *Phytophthora infestans* populations in East Asian countries. Phytopathology 84: 922–927.

Nishimura, R., K. Sato, W.H. Lee, U.P. Singh, C. Tun-tschu, E. Suryaningsih, S. Suwonakenee, P.Lumyong, C. Chamsawang, T. Wen-hua, S.K. Shrestha, M. Kato, N. Fujii, S. Akino, N. Kondo, K. Kobayashi and A. Ogoshi. (1999). Distribution of *Phytophthora infestans* Populations in Seven Asian Countries. Ann. Phytopathology. Soc. Jpn. 56: 66-75

Shaw, D.S.(1987). The breeding system of *Phytophthora infestans*: the role of the A2 mating type. In P.R. Day and G.J. Jellis (eds.), Genetics and Plant Pathogenesis. Blackwell Scientific Publications, Oxford, England, pp. 161-174.

Semal, J. (1995). The epic of potato blight (1845-1995). Cahiers Agric. 4: 287-298.

Smoot, J.J., F.J. Gough, H.A. Lamey, J.J. Eichenmuller and M.E. Gallegly. (1958). Production and germination of oospores of Phytophthora infestans. Phytopathology 48: 165-171.

Sujkowski, L.S., S.B. Goodwin, A.T. Dyer and W.E. Fry. (1994). Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. Phytopathology 84: 201-207.

(Received 30 October 2010; accepted 4 March 2011)